Data Summary and Review on the Acute Toxicity of AE C656948 (Fluopyram) Technical to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number {.....} EPA MRID Number 47372403

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	353315
	OECD Data Point	{.....}
	EPA MRID	47372403
	EPA Guideline	OPPTS 850.5400 (123-2)

Test material: AE C656948 (Fluopyram) Technical **Purity:** 94.7%
Common name Fluopyram
Chemical name: IUPAC N-[2-[3-Chloro-5-(trifluoromethyl)pyridine-2-yl]ethyl]-2-(trifluoromethyl)benzamide
CAS name N-[2-[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide
CAS No. 658066-35-4
Synonyms

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	080302	

CITATION: Banman, C.S. and C.V. Lam. 2007. Toxicity of AE C656948 Technical to the Green Alga,*Pseudokirchneriella subcapitata*. Unpublished study performed by Bayer CropScience, Research and Development Department, Stilwell, Kansas and sponsored by Bayer CropScience, Research Triangle Park, North Carolina. Laboratory Project ID: EBGMP048. Study completed March 30, 2007.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of the freshwater green algae *Pseudokirchneriella subcapitata* were exposed to AE C656948 (Fluopyram) Technical at nominal concentrations of 0 (negative and solvent controls), 0.102, 0.256, 0.64, 1.6, 4.0, and 10.0 mg a.i./L under static conditions. Mean-measured concentrations were <0.005 (<LOQ; controls), 0.093, 0.241, 0.584, 1.46, 3.78, and 9.53 mg a.i./L.

The percent inhibition in cell density, in the treated algal culture as compared to the negative control, ranged from 10 to 99%. The % inhibition in biomass, in the treated algal culture as compared to the negative control, ranged from 9 to 99%. The % inhibition in growth rate, in the treated algal culture as compared to the negative control, ranged from 2 to 95%.

The most sensitive endpoint was biomass, with NOAEC and EC50 values of 1.46 and 4.3 mg a.i./L, respectively.

Cell abnormalities were not reported.

This toxicity study is classified as scientifically sound or and {does or does not} satisfy the guideline requirement for a Tier II nonvascular plant toxicity study with the freshwater green algae, *Pseudokirchneriella subcapitata*.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}: 2.8 mg a.i./L 95% C.I.: 1.9 to 4.1 mg a.i./L
EC_{50}: 4.6 mg a.i./L 95% C.I.: 3.7 to 5.8 mg a.i./L
NOAEC: 1.46 mg a.i./L
Probit Slope: 7.63 ± 1.80

Biomass (Area under the growth curve)
EC_{05}: 2.6 mg a.i./L 95% C.I.: 2.0 to 3.2 mg a.i./L
EC_{50}: 4.3 mg a.i./L 95% C.I.: 3.8 to 4.9 mg a.i./L
NOAEC: 1.46 mg a.i./L
Probit Slope: 7.17 ± 1.12

Growth rate
EC_{05}: 3.7 mg a.i./L 95% C.I.: 3.1 to 4.3 mg a.i./L
EC_{50}: 6.0 mg a.i./L 95% C.I.: 5.5 to 6.5 mg a.i./L
NOAEC: 1.46 mg a.i./L
Probit Slope: 7.87 ± 0.653

Endpoint(s) Effected: Cell density, biomass, and growth rate

I. REPORTED MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following EPA FIFRA Guideline 123-2, OPPTS Guideline 850.5400 (2006 draft), and OECD Guideline 201 (2006).

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. Certificates of Analysis and Authenticity were also provided. This study was conducted in compliance with U.S. EPA FIFRA GLP standards (40 CFR Part 160).

A. REPORTED MATERIALS:

1. Test material AE C656948 (Fluopyram) Technical

Description: Beige powder

Lot No./Batch No.: 08528/0002 (Batch no.)

Purity: 94.7%

Stability of compound under test conditions: Time 0 measured concentrations yielded recoveries of 93 to 98% of the nominal test concentrations, and day 4 measured concentrations yielded recoveries of 88 to 95% of nominal, indicating that fluopyram technical was stable under the test conditions.

Storage conditions of test chemicals: Test chemicals were stored at ambient temperature.

Physicochemical properties of AE C656948 (Fluopyram) Technical.

Parameter	Values	Comments
Water solubility	**10 mg/L**	**Under algae testing conditions.**
Vapor pressure	**Not reported.**	
UV absorption	**Not reported.**	
pKa	**Not reported.**	
Kow	**Not reported.**	

2. Test organism:

Name:	Green algae, *Pseudokirchneriella subcapitata*
Strain:	Not reported
Source:	In-house cultures originally obtained from University of Texas, Austin, Texas, USA
Age of inoculum:	3 days
Method of cultivation:	Grown under test conditions **(**AAP medium) in an environmental chamber.

B. REPORTED STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was not conducted.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous. AAP medium Same as test. Not reported.
<u>Test system</u> Static/static renewal Renewal rate for static renewal	 Static. N/A
Incubation facility	Test vessels were placed on an orbital shaker table in a temperature-controlled environmental chamber.
Duration of the test	96 hours.
<u>Test vessel</u> Material: *(glass/stainless steel)*	 Glass

Parameter	Details
Size: Fill volume:	250 mL 100 mL
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	 7.3-7.4 7.6-10.0 Yes. $NaHCO_3$ N/A
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Distilled water. 7.5 ± 0.1 N/A Filter sterilized. Not reported. Not reported. Not reported. Not reported. Not reported.
Indicate how the test material is added to the medium (added directly or used stock solution)	A stock solution was prepared with test material and the solvent, which was then used to prepare the highest test solution concentration. Stock solution and algal media were prepared at the highest level and serially diluted to obtain the lower test concentrations.
Aeration or agitation	Agitation at 100 rpm

Parameter	Details
Initial cells density	1.0 x 10^4 cells/mL
Number of replicates Control: Solvent control: Treatments:	 3 3 3
Test concentrations Nominal: Measured:	 0 (Negative and solvent controls), 0.102, 0.256, 0.64, 1.6, 4.0, and 10.0 mg a.i./L <0.005 (<LOQ; controls), 0.093, 0.241, 0.584, 1.46, 3.78, and 9.53 mg a.i./L
Solvent (type, percentage, if used)	Dimethylformamide (0.10 mL/L)
Method and interval of analytical verification	Samples collected at 0 and 96 hours were analyzed using HPLC with UV (220 nm) detection.

Parameter	Details
Test conditions Temperature: Photoperiod: Light intensity and quality:	 23.4-24.3°C Continuous *Ca*. 4300 lux (400 footcandles) Cool-white fluorescent lighting
Reference chemical (if used) name: concentrations:	N/A
Other parameters, if any	None.

2. Observations:

Table 2: Observation parameters

Parameters	Details
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density, biomass (area under the growth curve), and growth rate
Measurement technique for cell density and other end points	Cell counts were conducted daily on all replicate vessels of each test concentration and the controls using a Z1 Beckman Coulter particle counter. Growth rate and biomass were determined using calculations that took into account time cell density from study initiation to termination.
Observation intervals	Every 24 hours.
Other observations, if any	None.

Parameters	Details
Indicate whether there was an exponential growth in the control	Yes. After 96 hours, the mean cell density was *ca*. 198.2x10^4 cells/mL in the negative control.
Were raw data included?	Yes.

II. REPORTED RESULTS:

A. REPORTED MORTALITY:

After 96 hours of exposure, cell density averaged 198.2 and 168.5 x 10^4 cells/mL in the negative and solvent controls, respectively, yielding inhibitions of 24.3, 13.8, 12.5, 2.9, 32.4, and 99.3% when compared to the pooled control at the mean-measured 0.093, 0.241, 0.584, 1.46, 3.78, and 9.53 mg a.i./L treatment levels, respectively.

Biomass (area under the growth curve) averaged 5987 and 5542cells · days/mL in the negative and solvent controls, respectively, yielding inhibitions of 18.3, 8.0, 15.8, 5.2, 39.3, and 99.3% when compared to the pooled control.

Growth rate averaged 0.055 and 0.053 $days^{-1}$ in the negative and solvent controls, yielding inhibitions of 5.0, 2.1, 1.7, 0.7, 7.3, and 94.5% when compared to the pooled control.

Observations at all test concentrations were normal.

B. REPORTED SUBLETHAL TOXICITY ENDPOINTS:

The study authors analyzed 96-hour cell density, and 72-hour and 96-hour biomass (area under the growth curve), and growth rate. The most sensitive endpoint for the 96-hour time period was biomass, with reported NOAEC and EC_{50} values of 1.46 and 4.07 (3.83-4.31) mg a.i./L, respectively. The most sensitive endpoint for the 72-hour time period was also biomass, with reported NOAEC and EC_{50} values of 1.46 and 3.97 mg a.i./L, respectively.

C. REPORTED STATISTICS: The study authors did not compare the negative control to the solvent control. The data were first tested for normality using Shapiro-Wilks' Test, and for homogeneity of variance using Levene's Test. If the data passed both tests, then data were analyzed using ANOVA and Dunnett's test. If the data did not demonstrate normality and homogeneity, an analysis of variance and a one-tailed Dunnett's test were used. The EC_{50} values and their confidence limits were determined by the logistic model or the Bruce/Versteeg cumulative normal model using nonlinear regression analysis. Statistics were conducted with SAS.

Table 3: Effect of AE C656948 (Fluopyram) Technical on algal growth (*Pseudokirchneriella subcapitata*)

Mean-measured and (Nominal) Concentrations (mg a.i./L)	Initial cell Density (x 10^4 cells/mL)	Cell density (x 10^4 cells/mL) at				
		24 hours	48 hours	72 hours	96 hours	
					cell count	% inhibition*
Negative control	1.0	4.15	31.52	118.18	198.24	N/A
Solvent control	1.0	4.12	30.50	115.56	168.52	15
0.093 (0.102)	1.0	4.09	30.21	96.10	138.76	30
0.241 (0.256)	1.0	3.96	31.22	110.22	157.99	20
0.584 (0.64)	1.0	3.93	28.09	93.50	160.51	19
1.46 (1.6)	1.0	3.91	28.46	109.88	177.98	10
3.78 (4.0)	1.0	3.55	16.46	67.30	123.91	37
9.53 (10.0)	1.0	1.26	1.26	1.75	1.33	99
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A	N/A

*Percent inhibition was calculated by the reviewer in comparison to the negative control.

Table 4: Effect of AE C656948 (Fluopyram) Technical on algal growth (*Pseudokirchneriella subcapitata*)

Mean-measured and (Nominal) Concentrations (mg a.i./L)	Initial Cell Density ($x10^4$ cells/mL)	Mean Growth Rate ($days^{-1}$)		Mean Biomass (Area Under the Growth Curve) (cells * days/mL)	
		0-96 Hours	Percent Inhibition*	0-96 hours	Percent Inhibition*
Negative Control	1.0	0.055	N/A	5987.2	N/A
Solvent control	1.0	0.053	3	5542.7	7
0.093 (0.102)	1.0	0.051	7	4710.8	21
0.241 (0.256)	1.0	0.053	3	5301.4	11
0.584 (0.64)	1.0	0.053	3	4854.5	19
1.46 (1.6)	1.0	0.053	3	5465.6	9
3.78 (4.0)	1.0	0.050	9	3498.4	42
9.53 (10.0)	1.0	0.003	94	37.48	99

*Percent inhibition was calculated by the reviewer in comparison to the negative control.

Table 5: Statistical endpoint values*

Statistical Endpoint	96-hour Cell Density	96-hour Growth rate	96-hour Biomass (Area under the growth curve)
NOAEC or EC_{05} (mg a.i./L)	3.78	3.78	1.46
LOAEC	9.53	9.53	3.78
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	4.26 (3.62-4.90)	9.5 (unreliable)	4.07 (3.83-4.31)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

*reported by the study authors, relative to the pooled control

Table 6: Statistical endpoint values*

Statistical Endpoint	72-hour	72-hour	72-hour

	Cell Density	**Growth rate**	**Biomass (Area under the growth curve)**
NOAEC or EC_{05} (mg a.i./L)	N/A	1.46	1.46
LOAEC	N/A	3.78	3.78
EC_{50} (mg a.i./L)	N/A	8.9	3.97
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	N/A	8.9 (unreliable)	3.97 (3.92-4.02)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

***** reported by the study authors, relative to the pooled control

III. REVIEWER'S EVALUATION

A. DEVIATIONS FROM GUIDELINES:

1. The physicochemical properties of the test material were not reported.
2. Pretest health of the test species was not reported.
3. The results of a periodic screening analysis of the dilution water and/or nutrient medium were not reported.
4. OPPTS guidelines require that the pH for this species stay within a range of 7.5 ± 0.1. The pH in this study was well above (range, 7.6-10.0) the recommended range at study termination.
5. The algae were only acclimated for 3 days; OPPTS guidelines recommend a 2-week acclimation period for algal cultures.

B. OTHER STUDY DEFICIENCIES:

None noted

C. VERIFICATION OF STATISTICAL RESULTS:
Statistical Method: Prior to determining the NOAEC values for cell density, area under the growth curve, and growth rate, the reviewer compared the negative and solvent control data for each endpoint using a Student's t-test, which indicated no significant differences between the negative and solvent controls. The reviewer tested the normality using the Chi-square and Shapiro Wilks tests and homogeneity of variance using the Hartley and Bartlett's test. If the data met the assumptions of ANOVA, the NOAEC values were determined using the parametric Williams' and Dunnett's tests. If the data did not meet the assumptions of ANOVA, the NOAEC values were determined using the non-parametric Steele's or Kruskal-Wallis test and visual interpretation of the data (cell density and biomass). The ECx values and probit slopes were determined using the probit analysis. All analyses were conducted using the mean-measured concentrations and Nuthatch statistical software.

Values input for cell density were the abbreviated values provided by the authors in the data tables, and are

mean to represent the value x 10^4 cells/mL. Values input for growth rate were multiplied by 10,000 to eliminate means with a zero value.

Cell density
EC_{05}: 2.8 mg a.i./L 95% C.I.: 1.9 to 4.1 mg a.i./L
EC_{50}: 4.6 mg a.i./L 95% C.I.: 3.7 to 5.8 mg a.i./L
NOAEC: 1.46 mg a.i./L
Probit Slope: 7.63 ± 1.80

Biomass (Area under the growth curve)
EC_{05}: 2.6 mg a.i./L 95% C.I.: 2.0 to 3.2 mg a.i./L
EC_{50}: 4.3 mg a.i./L 95% C.I.: 3.8 to 4.9 mg a.i./L
NOAEC: 1.46 mg a.i./L
Probit Slope: 7.17 ± 1.12

Growth rate
EC_{05}: 3.7 mg a.i./L 95% C.I.: 3.1 to 4.3 mg a.i./L
EC_{50}: 6.0 mg a.i./L 95% C.I.: 5.5 to 6.5 mg a.i./L
NOAEC: 1.46 mg a.i./L
Probit Slope: 7.87 ± 0.653

D. ADDITIONAL REVIEWER COMMENTS:

The study authors compared treatment levels with the pooled control, while the reviewer compared treatment response to the negative control; therefore, the reviewer's results are presented in the Conclusions and Executive Summary sections of this DER. Despite a different baseline comparison, the reviewer's conclusion that biomass was the most sensitive endpoint agreed with the study authors'.

The study authors conducted solubility trials to evaluate the maximum water solubility of fluopyram in the AAP medium. The authors used data from the study to determine the maximum test concentration for the definitive study.

The in-life portion of the study was conducted between August 14 and 18, 2006.

The NOAEC had to be visually determined for cell density and biomass due to the lack of sensitivity of the Kruskal-Wallis test. This statistical test did not detect (what the reviewer considers to be) the biologically significant reduction in these endpoints at the two highest treatment levels.

E. CONCLUSIONS: This study is/is not scientifically sound and is thus acceptable or unacceptable. The most sensitive endpoint was biomass, with NOAEC and EC_{50} values of 1.46 and 4.3 mg a.i./L, respectively.

Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}: 2.8 mg a.i./L 95% C.I.: 1.9 to 4.1 mg a.i./L
EC_{50}: 4.6 mg a.i./L 95% C.I.: 3.7 to 5.8 mg a.i./L
NOAEC: 1.46 mg a.i./L
Probit Slope: 7.63 ± 1.80

Biomass (Area under the growth curve)

EC_{05}:	2.6 mg a.i./L	95% C.I.: 2.0 to 3.2 mg a.i./L
EC_{50}:	4.3 mg a.i./L	95% C.I.: 3.8 to 4.9 mg a.i./L

NOAEC: 1.46 mg a.i./L
Probit Slope: 7.17 ± 1.12

Growth rate

EC_{05}:	3.7 mg a.i./L	95% C.I.: 3.1 to 4.3 mg a.i./L
EC_{50}:	6.0 mg a.i./L	95% C.I.: 5.5 to 6.5 mg a.i./L

NOAEC: 1.46 mg a.i./L
Probit Slope: 7.87 ± 0.653

Endpoint(s) Effected: Cell density, biomass, and growth rate

IV. REFERENCES:

1. American Public Health Association (APHA). 1989. Standard Methods for the Examination of Water and Wastewater, 17th edition. Washington, D.C.
2. ASTM. 2004. Standard Guide for Conducting Static 96-hour Acute Toxicity Tests with Microalgae. Standard E1218. West Conshohocken, PA.
3. Boutin, C., Freemark, K.E., and Keddy, C.J. 1993. Proposed Guidelines for Registration of Chemical Pesticides: Nontarget Plant Testing and Evaluation. Technical Report Series no. 145. Canadian Wildlife Service (Headquarters), Environment Canada, Ottawa.
4. Bruce, R.D. and D.J. Versteeg. 1992. "A Statistical Procedure for Modeling Continuous Data". Environmental Toxicology and Chemistry, Volume 11, pgs 1485-1494.
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7. SAS Institute. 1999-2001. PC-SAS version 8.2 (or greater). Cary, NC.
8. Stein, J.R. (ed.). 1973. Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press. Cambridge, England.
9. USEPA. 1982. Pesticide Assessment Guidelines, Subdivision J – Hazard Evaluation: Nontarget Plants. EPA-540/9-82-020. Office of Pesticide Programs. Washington, D.C. 55 pp.
10. USEPA. 1986. Standard Evaluation Procedure, Non-Target Plants: Growth and Reproduction of Aquatic Plants – Tiers 1 and 2. EPA-540/9-86-134. Office of Pesticides Programs. Washington, D.C.
11. USEPA. 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 54, No. 158: 34067-34074.
12. USEPA. 1994a. Pesticide Reregistration Rejection Rate Analysis. Ecological Effects. EPA 738-R-94-035: p. 161.
13. USEPA. 1994b. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA 600/4-91/002. Office of Research and Development. Cincinnati, OH.
14. USEPA. 1996. OPPTS 850.5400 *draft*: Algal Toxicity, Tiers I and II.
15. USEPA. 2006. OPPTS 850.5400 *draft*: Algal Toxicity.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
Fluopyram & P. subcapitata 96-hr Cell Density (mg/L)
File: 2403c        Transform: NO TRANSFORM

       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
-------------------------------------------------------------------------------
 GRP1 (SOLVENT CRTL) MEAN =   198.2367     CALCULATED t VALUE =     0.5584
 GRP2 (BLANK CRTL) MEAN   =   168.5233     DEGREES OF FREEDOM =     4
 DIFFERENCE IN MEANS      =    29.7133
-------------------------------------------------------------------------------
TABLE t VALUE (0.05 (2), 4) =  2.776    NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) =  4.604    NO significant difference at alpha=0.01


Fluopyram & P. subcapitata 96-hr Cell Density (mg/L)
File: 2403c       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
-------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
             _____     _____________     ___________    ___________    _____

EXPECTED     1.407         5.082             8.022          5.082      1.407
OBSERVED     0             7                 7              7          0

-------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   4.3919
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


Fluopyram & P. subcapitata 96-hr Cell Density (mg/L)
File: 2403c       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
-------------------------------------------------------------------------------

D = 25069.694

W =    0.946

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
-------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


Fluopyram & P. subcapitata 96-hr Cell Density (mg/L)
File: 2403c        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
-------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) = 4002041.26
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```
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.00

------------------------------------------------------------------------------

Data FAIL homogeneity test. Try another transformation.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).



Fluopyram & P. subcapitata 96-hr Cell Density (mg/L)
File: 2403c       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
------------------------------------------------------------------------------

Calculated B statistic =   29.14
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   6
------------------------------------------------------------------------------

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).


Fluopyram & P. subcapitata 96-hr Cell Density (mg/L)
File: 2403c        Transform: NO TRANSFORMATION

              KRUSKAL-WALLIS ANOVA BY RANKS  -  TABLE 1 OF 2
------------------------------------------------------------------------------
                              TRANSFORMED   MEAN CALCULATED IN      RANK
GROUP    IDENTIFICATION          MEAN         ORIGINAL UNITS         SUM
----- --------------------    -----------   ------------------  -----------
  1             Neg control     198.237           198.237          50.000
  2                   0.093     138.763           138.763          31.000
  3                   0.241     157.993           157.993          40.000
  4                   0.584     160.507           160.507          42.000
  5                    1.46     177.977           177.977          39.000
  6                    3.78     123.913           123.913          23.000
  7                    9.53       1.330             1.330           6.000
------------------------------------------------------------------------------

  Calculated H Value =  11.152         Critical H Value Table =  12.590
  Since  Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.



Fluopyram & P. subcapitata 96-hr Cell Density (mg/L)
File: 2403c        Transform: NO TRANSFORMATION

 DUNNS MULTIPLE COMPARISON -  KRUSKAL-WALLIS  -  TABLE 2 OF 2
```

```
------------------------------------------------------------------------
                                                    GROUP
                       TRANSFORMED  ORIGINAL   0 0 0 0 0 0 0
GROUP  IDENTIFICATION    MEAN        MEAN     7 6 2 3 4 5 1
-----  --------------  -----------  ---------  - - - - - - -
  7              9.53       1.330      1.330  \
  6              3.78     123.913    123.913  . \
  2             0.093     138.763    138.763  . . \
  3             0.241     157.993    157.993  . . . \
  4             0.584     160.507    160.507  . . . . \
  5              1.46     177.977    177.977  . . . . . \
  1       Neg control     198.237    198.237  . . . . . . \
------------------------------------------------------------------------

* = significant difference (p=0.05)            . = no significant difference
Table q value (0.05,7) =   3.038             SE =    5.066


Estimates of EC%
------------------------------------------------------------------------
Parameter   Estimate     95% Bounds        Std.Err.  Lower Bound
                       Lower     Upper               /Estimate
EC5              2.8       1.9       4.1     0.077      0.69
EC10             3.1       2.3       4.3     0.068      0.72
EC25             3.8       2.9       4.9     0.055      0.77
EC50             4.6       3.7       5.8     0.047      0.80

         Slope =     7.63  Std.Err. =     1.80


Goodness of fit: p =      0.54  based on DF=       4.0       14.
------------------------------------------------------------------------
2403C : Fluopyram & P. subcapitata 96-hr Cell Density (mg/L)
------------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
------------------------------------------------------------------------
     Dose     #Reps.     Obs.     Pred.      Obs.     Pred.   %Change
                         Mean     Mean     -Pred.   %Control

     0.00      3.00      198.      167.      31.5      100.      0.00
   0.0930      3.00      139.      167.     -27.9      100.  1.71e-14
    0.241      3.00      158.      167.     -8.70      100.  1.71e-14
    0.584      3.00      161.      167.     -6.19      100.  3.89e-10
     1.46      3.00      178.      167.      11.3      100.   0.00705
     3.78      3.00      124.      124.   -0.0151      74.3      25.7
     9.53      3.00      1.33      1.33   0.00120     0.797      99.2

Fluopyram & P. subcapitata 96-hr Biomass (mg/L)
File: 2403b        Transform: NO TRANSFORM

       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
------------------------------------------------------------------------------
 GRP1 (SOLVENT CRTL) MEAN =  5987.1600     CALCULATED t VALUE =     0.4539
 GRP2 (BLANK CRTL) MEAN   =  5542.6800     DEGREES OF FREEDOM =     4
 DIFFERENCE IN MEANS      =   444.4800
------------------------------------------------------------------------------
TABLE t VALUE (0.05 (2), 4) =  2.776    NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) =  4.604    NO significant difference at alpha=0.01

Fluopyram & P. subcapitata 96-hr Biomass (mg/L)
File: 2403b       Transform: NO TRANSFORMATION
```

```
Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     _____

EXPECTED     1.407         5.082             8.022           5.082       1.407
OBSERVED     0             7                 6               8           0

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   5.7230
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.



Fluopyram & P. subcapitata 96-hr Biomass (mg/L)
File: 2403b       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D = 9286508.909

W =    0.953

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.



Fluopyram & P. subcapitata 96-hr Biomass (mg/L)
File: 2403b        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) = 26377.77
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data FAIL homogeneity test. Try another transformation.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).



Fluopyram & P. subcapitata 96-hr Biomass (mg/L)
File: 2403b       Transform: NO TRANSFORMATION
```

```
Bartletts test for homogeneity of variance
------------------------------------------------------------------------------

Calculated B statistic =   17.18
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   6
------------------------------------------------------------------------------

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).


Fluopyram & P. subcapitata 96-hr Biomass (mg/L)
File: 2403b        Transform: NO TRANSFORMATION

              KRUSKAL-WALLIS ANOVA BY RANKS  -  TABLE 1 OF 2
------------------------------------------------------------------------------
                                TRANSFORMED   MEAN CALCULATED IN      RANK
GROUP    IDENTIFICATION           MEAN         ORIGINAL UNITS         SUM
----- --------------------    -----------   ------------------  -----------
  1            Neg control    5987.160           5987.160          53.000
  2                  0.093    4710.760           4710.760          33.000
  3                  0.241    5301.360           5301.360          44.000
  4                  0.584    4854.480           4854.480          35.000
  5                   1.46    5465.640           5465.640          43.000
  6                   3.78    3498.400           3498.400          17.000
  7                   9.53      37.480             37.480           6.000
------------------------------------------------------------------------------

  Calculated H Value =  13.939         Critical H Value Table =  12.590
  Since  Calc H > Crit H  REJECT Ho:All groups are equal.



Fluopyram & P. subcapitata 96-hr Biomass (mg/L)
File: 2403b        Transform: NO TRANSFORMATION

 DUNNS MULTIPLE COMPARISON -  KRUSKAL-WALLIS  -  TABLE 2 OF 2
------------------------------------------------------------------------------
                                                      GROUP
                      TRANSFORMED  ORIGINAL   0 0 0 0 0 0 0
GROUP  IDENTIFICATION    MEAN        MEAN     7 6 2 4 3 5 1
----- --------------- -----------  ---------  - - - - - - -
  7              9.53      37.480     37.480  \
  6              3.78    3498.400   3498.400  . \
  2             0.093    4710.760   4710.760  . . \
  4             0.584    4854.480   4854.480  . . . \
  3             0.241    5301.360   5301.360  . . . . \
  5              1.46    5465.640   5465.640  . . . . . \
  1       Neg control    5987.160   5987.160  * . . . . \
------------------------------------------------------------------------------

* = significant difference (p=0.05)           . = no significant difference
Table q value (0.05,7) =   3.038             SE =    5.066
```

```
 Estimates of EC%
--------------------------------------------------------------------------
Parameter   Estimate     95% Bounds        Std.Err.  Lower Bound
                       Lower     Upper               /Estimate
EC5              2.6       2.0       3.2     0.049      0.79
EC10             2.9       2.3       3.5     0.043      0.81
EC25             3.5       3.0       4.1     0.034      0.85
EC50             4.3       3.8       4.9     0.027      0.88

         Slope =     7.17  Std.Err. =     1.12


Goodness of fit: p =      0.38  based on DF=       4.0       14.
--------------------------------------------------------------------------
2403B : Fluopyram & P. subcapitata 96-hr Biomass (mg/L)
--------------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
--------------------------------------------------------------------------
     Dose     #Reps.     Obs.      Pred.      Obs.     Pred.   %Change
                         Mean      Mean     -Pred.   %Control

     0.00       3.00  5.99e+03  5.26e+03      723.      100.      0.00
   0.0930       3.00  4.71e+03  5.26e+03     -553.      100.  1.73e-14
    0.241       3.00  5.30e+03  5.26e+03      37.3      100.  1.73e-14
    0.584       3.00  4.85e+03  5.26e+03     -410.      100.  2.23e-08
     1.46       3.00  5.47e+03  5.26e+03      203.      100.    0.0355
     3.78       3.00  3.50e+03  3.50e+03    -0.978      66.5      33.5
     9.53       3.00      37.5      37.4    0.0976     0.710      99.3


Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g        Transform: NO TRANSFORM

       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
--------------------------------------------------------------------------------
 GRP1 (SOLVENT CRTL) MEAN =   547.3567     CALCULATED t VALUE =     0.6151
 GRP2 (BLANK CRTL) MEAN   =   529.0167     DEGREES OF FREEDOM =     4
 DIFFERENCE IN MEANS      =    18.3400
--------------------------------------------------------------------------------
TABLE t VALUE (0.05 (2), 4) =  2.776    NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) =  4.604    NO significant difference at alpha=0.01


 Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
             _____     _____________     ___________    ___________    _____

EXPECTED     1.407         5.082             8.022          5.082      1.407
OBSERVED     0             7                 7              7          0

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   4.3919
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.
```

```
Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D = 9721.272

W =    0.960

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.



Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =  209.56
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).



Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated B statistic =   11.43
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   6
--------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).
```

Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	634233.277	105705.546	152.231
Within (Error)	14	9721.272	694.377	
Total	20	643954.549		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	547.357	547.357		
2	0.093	511.143	511.143	1.683	
3	0.241	526.910	526.910	0.950	
4	0.584	528.843	528.843	0.860	
5	1.46	534.320	534.320	0.606	
6	3.78	498.880	498.880	2.253	
7	9.53	29.680	29.680	24.061	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.093	3	54.434	9.9	36.213
3	0.241	3	54.434	9.9	20.447
4	0.584	3	54.434	9.9	18.513
5	1.46	3	54.434	9.9	13.037
6	3.78	3	54.434	9.9	48.477
7	9.53	3	54.434	9.9	517.677

Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	547.357	547.357	547.357
2	0.093	3	511.143	511.143	525.304
3	0.241	3	526.910	526.910	525.304
4	0.584	3	528.843	528.843	525.304
5	1.46	3	534.320	534.320	525.304
6	3.78	3	498.880	498.880	498.880
7	9.53	3	29.680	29.680	29.680

Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)
File: 2403g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	547.357				
0.093	525.304	1.025		1.76	k= 1, v=14
0.241	525.304	1.025		1.85	k= 2, v=14
0.584	525.304	1.025		1.88	k= 3, v=14
1.46	525.304	1.025		1.89	k= 4, v=14
3.78	498.880	2.253	*	1.90	k= 5, v=14
9.53	29.680	24.061	*	1.91	k= 6, v=14

s = 26.351
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	3.7	3.1	4.3	0.034	0.85
EC10	4.1	3.6	4.8	0.030	0.87
EC25	4.9	4.4	5.5	0.024	0.89
EC50	6.0	5.5	6.5	0.017	0.92

Slope = 7.87 Std.Err. = 0.653

Goodness of fit: p = 0.60 based on DF= 4.0 14.

2403G : Fluopyram & P. subcapitata 96-hr Growth Rate (mg/L)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	547.	530.	17.6	100.	0.00
0.0930	3.00	511.	530.	-18.6	100.	2.15e-14
0.241	3.00	527.	530.	-2.80	100.	2.15e-14

0.584	3.00	529.	530.	-0.871	100.	8.58e-14
1.46	3.00	534.	530.	4.61	100.	7.19e-05
3.78	3.00	499.	499.	-0.000273	94.2	5.82
9.53	3.00	29.7	29.7	7.84e-06	5.60	94.4